SUMMARY

During this quarter, the following tasks were completed:

1) Modification of anti-contamination seals.

2) Laboratory evaluation of samplers with regard to their sterilizability by autoclaving, their aerodynamic particle-trapping efficiency, and their post-impact contamination characteristics.

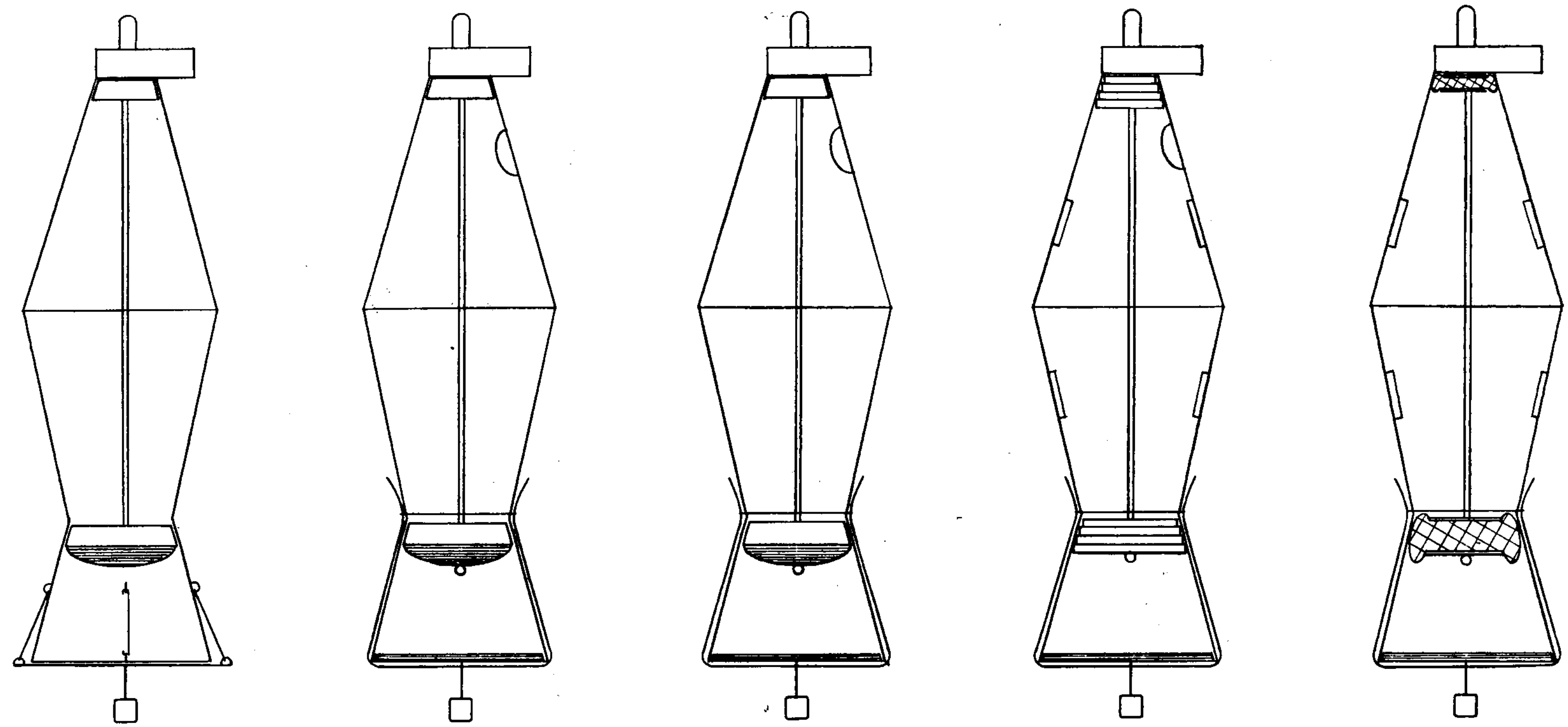
3) Successful preparation, launch, recovery, and analysis of the third flight under this contract.

4) Preliminary preparation for final flight scheduled in late January 1964.

MODIFICATION OF ANTI-CONTAMINATION SEALS

The basic concept of sampling the stratosphere for biological entities and the apparatus fabricated to accomplish this task have undergone relatively little change since the first prototype was flown in August 1962. Several minor modifications were introduced after the first flight (see Figure 1), but the basic hardware used (i.e., frames, spinnings, blowers, motors, flowmeters, filters) in all of the five flights to date is identical.

The main modifications introduced during the tenure of the present contract dealt with the anti-contamination seals. The original seals were metal pans fitted with polyurethane gaskets. These were replaced for the May 1963 flight with metal pans and silicone rubber seals. In July 1963, we employed a polyurethane plug made from overlapping sheets of Scottfoam filter. Although these were the best seals yet devised, we were not entirely satisfied that they provided the ultimate in post-impact protection. Therefore, for the November 1963 flight we developed a plug from non-adsorbent cotton wrapped in gauze--similar to those used in large-scale laboratory fermentation apparatus. These plugs were unaffected by autoclaving, pressure changes, or stratospheric temperatures, and they performed well during altitude chamber tests. Repeated trials showed that these "primitive" cotton plugs were as effective in minimizing post-impact contamination as any seals we had tried previously. They were chosen as the seal of choice for the final two flights under this contract.



August 1962	October 1962	May 1963	July 1963	November 1963
Seals: Metal pans polyurethane gaskets.	Dust covers: Held by nylon shroud cinched in place.	Seals: Metal pans with silicone rubber gaskets.	Seals: Plug made from overlapping sheets of polyurethane and seated against silicone rubber throat.	Seals: Gauze cotton plugs held in place with aluminum discs and seated against silicone rubber throat.
Dust cover: Aluminum sheet held in place with wires.	Pressure equilibration port protected with absolute filter.	Metal spinning replaces balsa wood nose cone.	Internal diffusion contamination controls introduced. Nose cones eliminated.	
Balsa wood nose cone over sealing pan.				

NASr-81 (August 1962 – October 1962) NASw-648 (May 1963 – November 1963)

Figure 1. Modifications in Stratospheric Biological Sampler (Original drawings presented in Final Report No. 2363, Contract NASr-81, 31 December 1962)

[redacted]

LABORATORY EVALUATION OF SAMPLERS

In the past, we had presumed that the autoclaving exposures used when preparing the samplers for a flight were adequate to sterilize the filters and the interior surfaces. It was recognized, however, that the unique configuration of the sampling instruments, and the wrapping procedure used might permit the formation of dead air spaces within the samplers which, in turn, would interfere with sterilization. Consequently, we fitted a sampler with thermocouples and recorded the interior temperature changes during several autoclaving sequences. We found that autoclaving at 120° C for one hour was sufficient to expose the sampler interior to 120° C for $>$ 30 minutes. Subsequent bacteriological analysis of the interior surfaces and filters showed that complete sterilization had taken place.

We fitted all of the samplers with clean filters and internal diffusion controls and, in the altitude chamber, simulated a sampling experiment using fluorescent particles. This was done to ascertain the relative number of particulates that would be trapped on the filter, as compared to those that would be entrained on the interior surfaces (i. e., on the diffusion controls) during the sampling process. This experiment was also designed to evaluate the aerodynamic characteristics of the sampler at reduced pressures, measuring the fluorescent particles deposited on the inlet cone, the sampler throat, and the cocked-open sealing plug. The results of this trial indicated that our sampler was aerodynamically efficient even at altitudes above 90 K, with relatively few micron-sized particles being entrained on surfaces other than the filter pad itself. These tests also verified the usefulness of the internal diffusion pads as reliable indicators of post-impact contamination.

Several trials were performed in which autoclaved samplers were sealed and dropped from a 10-ft height onto dusty ground that had been seeded with indicator contaminants. We verified to our satisfaction that the sealing mechanisms worked well and that we could minimize post-impact interior contamination to levels around 10 organisms per filter pad, even when the exterior contamination approximated 10^5 organisms/in.^{2}. Despite our best efforts, however, we could never quite achieve a perfectly germ-free sample after sealing and impacting. We decided, on the basis of repeated tests, that the noise level we would have to tolerate would be between 10 to 20 organisms per filter, as long as we would use this equipment and as long as the flights were programmed to impact on dust-generating soils. This contamination, however, would also be present on the interior diffusion controls and could be discounted by qualitative bacteriology if the stratospheric organisms were different from local soil types. Furthermore, since the sample volumes we intended to take were in the order of 10,000 to 100,000 ft^3 of ambient air, noise levels of 10-20 organisms would be significant only if the stratospheric counts were $<1 \times 10^{-3}$ to $<1 \times 10^{-4}$ organisms/ft^3 ambient air. At the very best, these flights would provide some maximum microbial limits for the stratosphere under given meteorological conditions.

FLIGHT NO. 3 (NASA 5)

On 30 October and 5 November, the pre-flight altitude chamber tests were carried out on the assembled payload. The necessary adjustments and re-calibrations were made, and the flight was scheduled for the week of 11 November. Inclement weather postponed the flight to the following week.

On 18 November a probe was launched from New Brighton, Minnesota. The total payload of 852 lb (including an atmospheric sampler being hitch-hiked for Dr. G. Soffen and Mr. J. Stuart of JPL) rose to 86,700 ft. The dust covers were jettisoned during ascent at 75,000 ft. The balloon was launched at 1312 GMT, attained maximum altitude at 1518 GMT, and started descent at 1554 GMT. Samplers 1 and 2 started collecting at this time. Sampler 1 ran for 6 min and collected 4,080 ft^3 at 86,000 ft; sampler 2 ran for 98 min and collected 97,170 ft^3 between 86 K and 60 K; sampler 3 ran for 24 min and collected 17,000 ft^3 between 60 K and 40 K. Sampler 4, which was hand-closed just prior to launch, served as a flight control.

The payload impacted in a manured barnyard at 1837 GMT near Wasau, Wisconsin. Inspection at impact site showed that all units, though thoroughly contaminated with barnyard soil on the outside, had locked and sealed themselves.

ANALYTICAL RESULTS OF FLIGHT NO. 3

Prior to launch, the balloon was dusted with a variety of fluorescent dusts, and after recovery the filter pads were carefully examined to determine what level of contamination may have originated from this source. We are now convinced that our sampler design and sampling program completely eliminate the balloon as a significant source of contaminants. This will permit us to utilize Sampler 1 (the float control) for another purpose during the next flight.

Bacteriologically, we found the following:

	Altitude	Volume Sampled	Total Count on Sampler Filter		Count on Internal Diffusion Controls	
Sampler 1	86 K	4,000 ft^3	1 mold	6 bacteria	4 mold	7 bacteria
Sampler 2	86 K - 60 K	97,000 ft^3	6 mold	11 bacteria	4 mold	20 bacteria
Sampler 3	60 K - 40 K	17,000 ft^3	1 mold	9 bacteria	6 mold	13 bacteria
Sampler 4	Control	---	0 mold	20 bacteria	0 mold	32 bacteria

These data suggest that the stratospheric contamination level during this probe was less than the noise level inherent in our sampling and analysis technique, as determined both by preliminary experiments and by the internal diffusion controls. Consequently, we are able to establish only maximum limits rather than actual values. According to these data, the contamination level between 90-60 K is $<2 \times 10^{-4}$ organisms per ambient cu ft, and the level between 60-40 K is $<1 \times 10^{-3}$ organisms per ambient cu ft. How much lower these limits can be established will have to await further improvements in the state-of-the-art: on the one hand, minimizing contamination to zero, and on the other hand, by designing experiments to acquire larger volumes of air.

It is significant that the contamination levels encountered during this flight were much lower than any previously encountered. We feel quite satisfied that the difficulties previously experienced with contamination control have been effectively resolved.

Qualitative identification of the organisms isolated from the filter pads and control pads revealed that most of the organisms from both sources were identical. The predominant flora were gram-positive bacilli, a few gram-negative rods, and several pigmented diphtheroids--in brief, typical soil flora. Of some interest, however, is the recovery of Cladosporium and Alternaria molds as the predominant filamentous fungi on the stratospheric samples. These genera were not found at all in the control sampler (#4).

We are quite pleased with this flight. It was a technical success, and the bacteriological data verify both the preceding flight and the flight of October 1962 but with a greater precision.

PREPARATIONS FOR COMING FLIGHT

We are preparing for a flight in late January 1964 which will be a replicate of this one with one modification: We will program a sampling sequence for these profiles, and will attempt once more to sample while passing through the tropopause. We do not intend to change anything on the sampling apparatus, and believe that we have reached a satisfactory noise level by controlling both pre-sampling and post-impact contamination. Further improvements would surely improve our precision, but the cost and time involved (i. e., air snatch; motor-driven closures; water recovery) would be beyond the scope of this contract.